Remarks:

Please find remarks directed towards the individual points of the examiner's detailed action of the March 24, 2006 office action itemized below. Only claim 52 of the November 23, 2004 amendment was addressed in the March 24, 2006 office action. These corrections will hopefully answer these. It is assumed that the remainder of the November 23,2004 amendment, including the substitute specification, and the remarks addressing the prior art issue, continue to be examined.

Change of Invention:

The examiner objects to certain terms used in the major independent claim (claim 52) submitted with the November 23, 2004 amendment. These terms are not adequately defined, to the examiner's satisfaction, in the (original or substitute) specification. The existence of these terms and their use in method steps of the major independent claim, make these new claims describe a new or changed invention. Changing an invention after the initial submission is not allowed therefore, the entire amendment is regarded as not fully responsive. The comments below address the issue of the previous use of terms that are not present in the specification, and the steps taken here to correct this.

Use of: "ligand standard series":

The term used which is most objected to is that of "ligand standard series" The term "ligand standard series" was essentially mentioned in the original and substitute specification. With the latter, the passage on page 14, line 2 of the specification describes this:

> The assay was **standardized** by loading a **series** of treated solutions of known amounts of pure FITC-Tf onto said electrophoresis gel. These consisted of 8 samples applied so that 2.4, 4.8, 9.7, 19, 39, 78, 156, and 313 ηg FITC-Tf protein were delivered per well, respectively.

This was thought to define a ligand standard series to those experienced in the art. In this context, FITC-Tf was the ligand being used. In addition, in the original and current abstract of the disclosure, the term "hapten-ligand standards" was mentioned on page 25, line 8 of the specification:

> Known amounts of hapten-ligand are similarly applied to the membrane, to provide for hapten-**ligand standards**.

Thus, it was thought that the use of "ligand standard series" entailed the use of terms covered in the specification. Also, these terms are not novel, in general, and are easily understood by those experienced in the art. However, since the term "ligand standard series" was not mentioned *per se* in the specification, it has been omitted from the claims, and replaced with the term "standards".

Use of: "standards":

The term "standards" is described throughout the original specification, and the substitute specification (submitted with the November 23, 2004 amendment). In the specification submitted November 23, 2004, the use of standards, and/or a definition of standards, and/or a teaching of their make and use, is cited in the following passages (the section on Page 20, line 18, teaches the particular manufacture of those standards used):

Page 11, line 13:

In Figure 1G, both samples are loaded onto an electrophoresis gel, along with standards containing increasing levels of known amounts of FITC-Tf.

Page 12, line 5:

The amount of FITC-Tf in this band can be estimated by comparing its signal to that of the standards.

page 14 line 2:

The assay was standardized by loading a series of treated solutions of known amounts of pure FITC-Tf onto said electrophoresis gel. These consisted of 8 samples applied so that 2.4, 4.8, 9.7, 19, 39, 78, 156, and 313 ηg FITC-Tf protein were delivered per well, respectively.

Page 16, line 22:

In Figure 3G, both samples are loaded onto an electrophoresis gel, along with standards containing increasing levels of known amounts of FITC-Annexin V.

Page 17, line 14:

The amount of FITC-Annexin V in this band can be estimated by comparing its signal to that of the standards.

Page 18, line 12:

Also run on the same gel were four pure FITC-Annexin V standards of 0.5, 1, 2, and 4 μg Annexin V protein per well.

Page 19, line 18:

Also run on the same gel were four pure FITC-Con A standards consisting of 1, 2, 4, and 8 ηg total FITC-Con A protein loaded per lane, respectively.

Page 20, line 18:

For the standards, increasing volumes (2, 4, 8, and 16 μL) of a 100 ηg/ml FITC-Con A solution were applied to a nitrocellulose membrane.

Page 22, line 9:

Also run on the same gel were four pure FITC-Avidin standards of 100, 200, 400, and 800 ρg total FITC-Avidin protein loaded per lane, respectively.

Page 24, line 2:

Also run on the same gel were three treated pure FITC-Insulin standards of 1, 2, and 4 ηg total FITC-Insulin protein loaded per lane, respectively.

Page 24, line 8:

Increasing signal is returned for increasing loads of FITC-insulin in the standards (Figure 8, lanes 1-3).

Page 26, line 5:

Results obtained from the standards are used to construct a standard curve which is then used to calculate the amount of hapten-ligand in the membrane areas corresponding to the unknowns. Thus, the amount of hapten-ligand originally bound to the surface can be determined.

Page 26, line 12:

The procedure allows for easy standardization as different user-definable levels of a standard solution of the Hapten-ligand can be simultaneously applied to the electrophoresis gel or to the dot-blot or slot-blot membrane

Page 27, line 8 (Abstract of Disclosure):

Known amounts of hapten-ligand are similarly applied to the membrane, to provide for hapten-ligand standards.

Page 27, line 13 (Abstract of Disclosure):

Results obtained from the standards are used to calculate the amount of hapten-ligand in the membrane areas corresponding to the unknowns. Thus, the amount of hapten-ligand originally bound to the surface can be determined.

In addition to mentioning and defining the term "standards", the following passages delineate a more complete teaching of how to use information from the standards to determine the amount of labeled (hapten) ligand in the unknowns:

Page 18, line 17:

Figure 4B displays the standard curve obtained when the density of the bands obtained from the image analysis of the standards from Figure 4A were plotted against the amount of Annexin-V present in each standard band. The equation shown on the curve was used to calculate the amount of Annexin-V present in the bands from the cell lysates, thus enabling the determination of Annexin-V bound per cell equivalent (or cell protein) for the various treatments (Figure 4C).

Page 20, line 3:

Figure 5B displays the standard curve obtained when the density of the bands obtained from the image analysis of the standards from Figure 5A were plotted against the amount of FITC-Con A present in each standard band. The equation shown on the curve was used to calculate the amount of Con A present in the bands from the cell lysates, thus enabling the determination of Con A bound per cell equivalent for the various treatments, as shown in Figure 5C.

Page 21, line 5:

Figure 6C displays the standard curve obtained when the density of the bands obtained from the image analysis of the standards from Figure 6A were plotted against the amount of Con A present in each standard dot. To maintain linearity, only the first three were used. The equation shown on the curve in Figure 6C was used to calculate the amount of Con A present in the dots from the cell lysates in Figure 6B, thus enabling the determination of Con A bound per cell equivalent for the various treatments, as shown in Figure 6D.

Page 22, line 14:

Figure 7B displays the standard curve obtained when the density of the bands obtained from the image analysis of the standards from Figure 7A were plotted against the amount of FITC-Avidin present in each standard band. The equation shown on the curve was used to calculate the amount of FITC-Avidin present in the bands from the cell lysates, thus enabling the determination of FITC-Avidin bound per cell equivalent for the various treatments, as shown in Figure 7C.

Name of invention in major claim:

The examiner objects to the description of the method in the major independent claim in the November 23, 2004 amendment (claim 52). Here, the method is described as : "A method for the measurement of biological ligand association with an insoluble surface, comprising: ... " However, the initial major claim for this invention described the method as : "A new method for the evaluation of biological ligand binding using non-radioisotopic immunologically recognizable hapten-conjugated ligands." The examiner indicates that the alteration in the description of the method from the original to the current is cited as a changing of the invention, and therefore makes the amendment as not fully responsive.

The abstract of the disclosure is the same now as it was in the originally submitted specification. The abstract of the disclosure states the invention as (This is repeated in the conclusion paragraph, on page 25, line 15.):

> The invention is a procedure for measuring the binding of an entity (ligand) to a surface by using a hapten-conjugated version of the ligand (hapten-ligand).

The method stated in claim 52 of the November 23, 2004 amendment was made to more clearly reflect the method stated in the abstract. The inventor did not feel that this was significantly different from the original stating of the method. In retrospect, it includes the word "association", which is not in the specification, also,

the dropping of the "hapten-conjugated ligands" phrase perhaps caused it to be too broad.

The method stated as "A new method for the evaluation of biological ligand binding using non-radioisotopic immunologically recognizable hapten-conjugated ligands", does not truly reflect that in the abstract. It contains the phrase "non-radioisotopic", which is an unnecessary negative. It states what the invention is not rather that what it is. It also contains the term "biological", which is a limitation that is not stated in the abstract of the disclosure, nor in the conclusion. Thus, the claim containing this method description, was canceled..

The specification refers to the invention as broader than "biological", in a number of places. For example, on. page 7, line 5:

> The ligand could be a growth factor or any other factor whose study involves the need for persons to assess the ability of cells, or any other insoluble particle or material, to bind it.

On page 8, line 15:

> This invention not only offers a novel non-radioactive method for assessing ligand binding to cell surfaces, but can be used to quantitate the binding of any recognizable hapten-containing binding factor to any surface ...

On page 24, line 15:

> General applications: The assay strategy can apply to any ligand ...

On page 25, line 15:

> Conclusion: this new method is a procedure for measuring the binding of an entity (ligand) to a surface....

And in the abstract of the disclosure:

> The invention is a procedure for measuring the binding of an entity (ligand) to a surface

However, since the group for this invention is: the evaluation of growth factors or biological factors (group I?, class 435, subclass 7.9), the inventor is not sure that the term "biological" must be included in the statement of the method, in the major independent method claim.

The preferred statement of the method is: "A new method for the evaluation of ligand binding using recognizable hapten-conjugated ligands". This best describes the invention to that as mentioned in the specification.

Amendments to the Claims:

An examination of the claims in regards to the objection to the "ligand standard series" phrase revealed other terms in the claims which were likewise not referred to adequately in the specification. So, all claims were amended so as to include

only those methods and terms which are contained in the specification. This process produced amended claims that were undesirably marked up, therefore, new claims were drafted. These claims closely match those submitted on November 23, 2004, in content. The order is slightly different, so that dependent claims follow a logical progression. The terms used in the claims, and the location of their sources in the specification are:

Claim 75:

The term: hapten-ligand is defined on page 7, line 10:

> The invention makes use of many available anti-hapten antibodies which specifically recognize a hapten-conjugated binding entity or ligand (**hapten-ligand**) in a complex mixture of other compounds which are naturally devoid of the hapten.

The term binding surface, is defined on page 24, line 19:

> Any particles or other insoluble material can serve as the **binding surface.**

A known quantity of a binding surface is referred to in various places, including page 12, line 17 (where the cells are the binding surface):

> The cell number in three wells was determined ...

This leads to page 15, line 4:

> Here, all lanes were loaded with lysates from **equal quantities** of cells initially exposed to the concentration of FITC-TF listed above the blot.

The terms standards and known amounts of hapten-ligand are defined on page 27, line 8:

> **Known amounts of hapten-ligand** are similarly applied to the membrane, to provide for hapten-ligand **standards**.

The term bound ligand, is defined on page 24, line 21:

> The current method requires that the **bound ligand** be removed from the binding surface so that it can be

Reference to ligand not bound to the binding surface is made numerous times, as "unbound samples". The term unbound ligand, is defined on page 24, line 20:

> Centrifugation and re-suspension of suspended particulate binding substrates would serve as a method for washing those of **unbound ligand.**

And on page 25, line 17:

> An excess of the hapten-ligand is presented to the binding surface and excess (**unbound**) hapten-**ligand** is washed off

The terms solubilized mixture, and applying onto a membrane support, are defined on page 7, line 15:

> After binding, excess hapten-ligand is washed off, and all bound hapten-ligand is solubilized with or without solubilized substrate components. The **solubilized mixture** is **applied to a membrane support** directly or is separated by electrophoresis and then applied to a membrane support.

The terms: membrane-bound hapten-ligand, and a reference to detecting, are found on page 7, line 17:

The included **membrane-bound hapten-ligand** is **detected** by treatment of the membrane ...

The term signal defined, on page 8, line 4:

The two-antibody incubation steps amplify the **signal** so that in combination with enhanced chemi-luminescence, very low levels of hapten-ligand can be detected.

This "signal" refers to the "color or light" on page 7, line 19:

The amount of resultant membrane-associated localized enzyme is determined by incubation with a color or light-producing substrate for that enzyme...

Claim 76

The term specific binding is defined on page 12, line 9:

Thus, competition for Tf binding to the cells between FITC-Tf and Tf is seen, demonstrating **specific binding** to the cells by the FITC-Tf.

And on page 20, line 8:

Cells which were initially treated with both FITC-Con A and un-conjugated Con A displayed markedly lower binding of FITC-Con A than cells which received FITC-Con A only, indicating competition for binding between FITC-Con A and unconjugated Con A, further indicating **specific cell binding** by the FITC-Con A.

Claim 77

The terms separated unknowns, and separated standards are mentioned on page 9, line 5:

> Figures 2A-2F show reproductions of actual enhanced chemiluminescence films of electrophoretically **separated unknowns** and **standards**,

The term separating, is referred to on page 27, line 6:

> Bound hapten-ligand is then solubilized (removed) and applied to a membrane support or **separated** ...

Claim 78

The term electrophoresis, is mentioned many times, including on page 7, line 15:

> The solubilized mixture is applied to a membrane support directly or is **separated by electrophoresis** and then applied to a membrane support.

Claim 79

The phrase: "said electrophoresis method is selected from the group consisting essentially of sodium dodecyl sulfate polyacrylamide electrophoresis, electrophoresis according to Schagger Von Jagow, and agarose electrophoresis", is supported by multiple passages: on page 13, line 13 (defines SDS-PAGE):

> **sodium dodecyl sulfate-polyacrylamide gel electrophoresis** (SDS-PAGE)

On page 13, line 16:

Treated samples (150 uL each) were loaded onto an acrylamide **SDS-PAGE** electrophoresis gel.

On page 19, line 11:

supernatants were treated for (...), and separated by **SDS-PAGE.**

On page 23, line 18:

Lysates (100 μl each) and aliquots of the treated unbound samples were separated by SDS-PAGE run according to **Schagger-Von Jagow.**

And on page 25, line 8:

After binding, the bound labeled DNA is released through heat de-naturation, is separated by **agarose electrophoresis**, electro-blotted to nytran,

Claim 80:

The term "enzyme-conjugated antibody to the hapten" is referred to on page 25, line 22:

The membrane-bound hapten-ligand is detected by application of an **enzyme-conjugated antibody to the hapten ...**

Claim 82:

The term "anti-hapten antibody" is referred to on page 7, line 17:

The included membrane-bound hapten-ligand is detected by treatment of the membrane with **anti-hapten antibody**

The term "enzyme-conjugated antibody to anti-hapten antibody" is referred to on page 26, line 1:

or by application of an antibody to the hapten followed by application of an **enzyme-conjugated antibody to the anti-hapten antibody.**

Claims 81 and 83:

The term horse radish peroxidase(HRP) is used throughout the specification, and is defined on page 14, line 15:

The membrane was incubated with 1:2000 **horse radish peroxidase**-conjugated goat anti-rabbit IgG in block solution for 2h at 25°C and washed again.

Claim 84

It was thought that the use of the term "cells" would be too broad (ambiguous). The term "biological cells" is not used *per se*, however, the term "biological cells" is referred to in context on page 7, line 3.

The present invention relates to the need in **biological** research to measure the ability of **cells** ... to bind

And on page 11, line 4:

Specifically, the method developed measures the binding of transferrin, concanavalin-A, avidin, annexin-V, and insulin **to cell** surfaces.

Claim 85

The phrase: "compounds which can be specifically recognized by an antibody" , is cited on page 24, line 15:

General applications: The assay strategy can apply to any ligand conjugated with a **compound which can be specifically recognized by an antibody**.

Claim 86

The term "fluorescein" (or FITC) is used throughout the specification. The phrase: "wherein said hapten is selected from the group consisting essentially of, fluorescein, biotin, rhodamine, and digoxygenin", is referred to on. page 24, line 16:

In particular, anti-**digoxygenin**, anti-**rhodamine** and anti-**biotin** antibodies exist which would recognize ligands conjugated with those compounds.

Claim 87

The phrase: "wherein said biological ligand is a biological factor, is supported on. page 7, line 5:

The ligand could be a growth factor or any other **factor** whose study involves the need for persons to assess the ability of cells, or any other insoluble particle or material, to bind it.

Claim 88

The phrase: "wherein said biological ligand is a protein, is supported on. page 8, line 11:

In particular, the method lends itself to the measurement of hapten-conjugated **protein** binding to cell and tissue surfaces.

Claim 89

The phrase: "wherein said protein is selected from the group consisting essentially of transferrin, concanavalin A, avidin, annexin V, and insulin", is referred to on page 11, line 4:

Specifically, the method developed measures the binding of transferrin, concanavalin-A, avidin, annexin-V, and insulin to cell surfaces.

Claim 90

The phrase: "wherein said biological ligand is DNA, is supported on. page 25, line 7:

In another embodiment, the assay could be used to verify the hybridization of a known biotin-labeled **DNA** to a surface.

Claim 91

The term "blotting" is cited on page 11, line 17:

> These are **blotted** onto a membrane as shown in Figure 1I

And on page 17 line 13

> These are **blotted** onto a membrane as shown in Figure 3I, where their relative positions are maintained.

Claim 92:

The definition and/or mention of the terms: "electroblotting, dot blotting, slot blotting, or Western blotting", occurs in multiple places: On page 4, line 7:

> **Western blotting** is a technique where cell lysates obtained by detergent treatment are **separated by electrophoresis** and the separated components contained within the electrophoresis gel are **driven onto a protein-binding membrane** via electric current.

On page 4, line 15:

> **Dot-blotting or slot-blotting** is where the cell lysate is applied directly to a binding-membrane without prior separation by electrophoresis.

On page 20, line 13:

> The replacement of electrophoresis with **dot-blot** techniques is possible

On page 25, line 8:

After binding, the bound labeled DNA is released through heat denaturation, is separated by agarose electrophoresis, **electro-blotted** to nytran,

On page 26, line 13:

the Hapten-ligand can be simultaneously applied to the electrophoresis gel or to **the dot-blot or slot-blot** membrane

On page 27, line 6:

Bound hapten-ligand is then solubilized (removed) and applied to a membrane support or **separated by electrophoresis and applied to a membrane** support

Claim 93:

The phrase "wherein said membrane support includes conventional transfer membranes." Refers to page 25, line 1:

It must also bind to a **conventional transfer membrane** for detection with the antibody.

Claim 94:

The claim element: "membrane is selected from the group consisting essentially of protein binding membranes, and nucleic acid binding membranes" is not stated directly. However, it can be logically inferred, since the ligand can be protein or DNA (claim 81), and must bind to a membrane support (page 27, line 6, for claim 92).

Claim 95:

The phrase "membrane support is selected from the group consisting essentially of nitrocellulose, or nytran", is supported on page 14, line 7:

> A 14 X 14 cm **nitrocellulose** membrane was... Gel components were transferred to the membrane at a constant voltage of 40 V for 1.5h.

And on page 25, line 9:

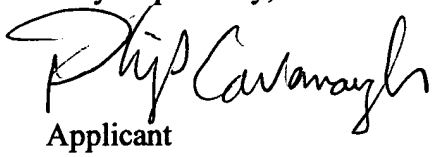
> electro-blotted to **nytran**, and is detected

The majority of the techniques used in this invention are widely known in the prior art. Multiple forms of electrophoresis have been in wide use for decades. Multiple methods to transfer components (from electrophoresis gels or elsewhere) to membranes are likewise known, and practiced routinely throughout the World. Specific mention of specific examples of these are claimed. However, considering the widespread knowledge, the broadest of claims entailing the use of these techniques are the most logical. Thus, it seems as though the broad claims of electrophoresis, blotting, and conventional membranes are the most appropriate. Particularly since two methods of blotting, two methods of electrophoresis, and two different cell systems (adherent and in suspension) were described in the specification.

Request for constructive assistance

The applicant has amended the specification, drawings, and claims so that they are proper and define a novel non-obvious method. If this application is not believed to be in condition for allowance, the applicant respectfully requests the constructive assistance and suggestions of the examiner pursuant to MPEP 2173.02 and 707.07(j), in order so that the applicant can submit an allowable application as soon as possible.

Very respectfully,



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